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Comparison of ITS sequences from UK and North American sugar-beet powdery mildews and the designation of *Erysiphe betae*

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ABSTRACT

Powdery mildew of sugar beet, a disease of major economic significance, was first described at the beginning of the 20th century, and since then there has been some confusion over the correct taxonomic identity of the causal agent. In Europe, the fungus was initially classified as the novel species *Microsphaera betae*, later re-named *Erysiphe betae*, whilst in America it was identified as *E. polygoni*, despite sugar-beet isolates from both regions having a host range restricted to *Beta* species. It is possible that more than one fungus causes the disease, as published descriptions of conidiogenesis have differed. In this study, isolates of the fungus collected from sugar beet in the UK and USA were investigated for polymorphisms in the rDNA ITS region to determine if the same species caused the disease in both countries, whether there was any justification for the retention of the name *E. polygoni* in the USA, and to search for evidence of a second species infecting sugar beet. From a total of 18 isolates examined, 23 ITS sequences were obtained. Fifteen of these, which included the UK and USA isolates, were identical and the remainder had single-base substitutions, indicating that the fungi were conspecific. Dendrogram analysis of *Erysiphales* ITS regions revealed that the UK and North American isolates were more closely related to *E. heraclei* than to *E. polygoni*. It is proposed that the species name *Erysiphe betae* be used for the powdery mildew fungus that infects sugar beet. No evidence was found in this study for a second sugar-beet powdery mildew species.

Introduction

Infection of sugar beet (*Beta vulgaris* subsp. *vulgaris*, *Amaranthaceae*, formerly *Chenopodiaceae*) by powdery mildew is economically significant for growers worldwide and can cause sugar yield losses of up to 30 % (Francis 2002). Classifying the causal agent of this disease has been difficult because, in some countries, the fungus was only present in its anamorph stage at the time it was described. Despite a century of study, the nomenclature of sugar-beet powdery mildew is still not fully standardised in the literature.

The disease was first described in Europe during the early 20th century (Vañha 1903) and it steadily spread through the continent, being recorded in the UK in 1935 (Anon. 1936),

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then reaching the USA two years later (Yarwood 1937). Vañha (1903) named the causal fungus *Microsphaera betae* based on chasmothecium (i.e. cleistothecium) appendage morphology and other characteristics. However, in the USA, sugar-beet powdery mildew was initially identified as a form of *Erysiphe polygoni* based on conidiophore morphology, in the absence of any chasmothecial samples (Yarwood 1937). Weltzien (1963) re-classified European isolates as a new species, *E. betae*, after an extensive biometrical study using a large number of chasmothecial samples. In the USA, powdery mildew re-appeared as a major problem on sugar beet in the 1970s, this time producing chasmothecia. The fungus was again classified as *E. polygoni* by Coyier *et al.* (1975) as the size of the chasmothecia fell within the range previously reported for *E. polygoni* and they did not consider that host range was a sufficient criterion for speciation.

The value of host-range information in classifying powdery mildew fungi has been controversial (Adam *et al.* 1999), although it can be used for taxonomic purposes (Braun 1995; Braun *et al.* 2002). Vañha (1903) used host-range information in his original description of sugar-beet powdery mildew. Weltzien (1963) noted that *E. polygoni* had not previously been reported on any member(s) of the *Chenopodiaceae*, so argued that it was unlikely to be the cause of sugar-beet powdery mildew, although he did not perform any host range experiments with the sugar-beet isolates. Positive proof of the narrow host-range of sugar-beet powdery mildew (from Europe) was gained when the fungus was test-inoculated onto 52 crop, weed and wild halophytic species (Drandarevski 1969). The results showed that the beet powdery mildew fungus was restricted to *Beta* species. Similarly, in the USA, test inoculations of powdery mildew isolates from sugar beet onto 33 different plant species showed that the fungus could only infect, and sporulate, on sugar beet (Ruppel & Tomasovic 1977). In addition, an *E. polygoni* isolate collected from *Rumex crispus* growing in a sugar-beet field could not infect sugar beet.

Further studies have since identified *Chenopodium ambrosoides*, *C. anthelminticum* and *C. botrys* in Asia (Braun 1987; Otani 1988; Nomura 1997) and *Celosia* sp. (*Amaranthaceae*) in Germany (Braun 1998) as hosts of *E. betae*, based on studies of chasmothecium morphology and other characters.

As well as morphological and host-range data, a third characteristic useful in the taxonomic classification of obligate biotrophic plant pathogens such as powdery mildew fungi is DNA sequence variation. Many studies have successfully used rDNA ITS sequence polymorphisms (White *et al.* 1990) to differentiate *Erysiphales* species and study their phylogeny (Takamatsu *et al.* 1998; Takamatsu *et al.* 1999; Saenz & Taylor 1999; Mori *et al.* 2000; Kiss *et al.* 2002, 2005; Cunnington *et al.* 2003). PCR amplification of the ITS region is a technique admirably suited to obligate pathogens because of its success despite the often very small amounts of template DNA that are available. Saenz & Taylor (1999) included, amongst many other species, *E. betae* and *E. polygoni* in their dendrogram analyses and revealed the two species to be closely related, but not necessarily conspecific.

A further line of investigation that needs developing is whether more than one powdery mildew fungus can infect sugar beet. In details of UK isolates given by Hull (1949, 1971), sugar-beet powdery mildew is described as producing long chains of conidia (catenate conidiogenesis). This is contradictory to the short chains or single conidia reported elsewhere (Weltzien 1963; Drandarevski 1969; Mukhopadhyay & Russell 1979; Francis 2002). It has also been suggested that the single case of the disease observed in the 1930s in the USA might have been due to a different species than that which caused an epiphytotic there in 1974 (Weltzien 1978). In the case of tomato powdery mildew, descriptions of conidiogenesis were contradictory and this led to the discovery that two species, *Oidium lycopersici* and *O. neolycopersici*, acted as causal agents (Kiss *et al.* 2001). If more than one powdery mildew species infects sugar beet, this would create complications in powdery mildew resistance breeding programmes (Francis 2002; Francis & Luterbacher 2003). *Arabidopsis* can be infected by two powdery mildews, *E. cruciferarum* and *Golovinomyces cichoracearum*, and large-scale testing has revealed that accessions contain resistance to both, neither, or either one of the fungi (Adam *et al.* 1999; Vogel & Somerville 2002). Investigation of ITS polymorphisms is a powerful technique for resolving whether more than one powdery mildew is present. In a recent study, Cunnington *et al.* (2004) used ITS sequence data to discover a previously-unknown powdery mildew taxon on *Delphinium*, and Takamatsu *et al.* (2002) were able to distinguish *E. glycines* and *E. diffusa*, two species that can simultaneously infect soybean but have virtually identical anamorphs.

In this study, isolates have been taken as either cellulose acetate peels from the surfaces of sugar-beet leaves, or as spores vacuumed or rinsed from leaf surfaces, and are thus parts of powdery mildew colonies. Sequences of the ITS regions of UK and North American sugar-beet powdery mildew isolates were determined, compared with each other, and with other *Erysiphales* ITSs deposited in the sequence databases (including sequences deposited since Saenz & Taylor's 1999 study), to determine: (1) whether there were any differences between the UK and North American isolates; (2) whether the name *E. betae* or *E. polygoni* was most suitable for the fungus; and (3) whether there was any evidence for a second, cryptic, powdery mildew fungus on sugar beet in either region.

Materials and methods

Collection of isolates and DNA extraction

Sugar-beet leaves that were heavily infected with powdery mildew, but not senescent or exhibiting symptoms of other pests or diseases, were used as sources of the fungus. For the UK isolates, mycelium was peeled from leaf surfaces by painting the leaves with a 'glue' of cellulose acetate dissolved in acetone, which was then stripped from the leaves, along with the mycelium, after the acetone had evaporated (Whitehouse *et al.* 1982; Francis 1996). This process avoids contaminating the fungal sample with host plant DNA. The acetate 'peels' were immediately frozen in liquid nitrogen, ground with DNase-free sand in microcentrifuge tubes, and DNA was extracted from the resultant powder using a Nucleon Phytopure kit (GE Healthcare, Chalfont St Giles, UK). DNA from North American isolates was extracted from mycelium

and spores collected either by vacuuming air-dried infected leaves (for the non-Fargo isolates) or rinsing symptomatic leaves with 0.1 % Tween 20 into a microcentrifuge tube and concentrating the spores by centrifugation (for the Fargo isolate). For both types of samples, the spore/mycelium mixture was added to a mortar and processed as previously reported (Weiland 1997). All DNA samples were treated with RNase A before PCR. The origins of the samples used in this study are detailed in Table 1.

Acquisition and analysis of ITS sequence data

Because *Erysiphe betae* cannot be grown in axenic culture and spores/mycelium taken directly off infected leaves were used as the source for template DNA, it was possible that the PCR step could amplify products from other phylloplane organisms associated with the powdery mildew colonies (Bridge *et al.* 2003). PCR was performed according to standard methods with 1 ng template DNA using primers ITS4 and ITS5 (White *et al.* 1990) to amplify the ITS region and then, instead of directly sequencing the PCR product, the following further procedures were carried out to check for contaminating PCR products. PCR products were cloned using a pGEM-T Easy Vector kit (Promega, Southampton) and plasmid DNA was purified from randomly-selected recombinant clones using standard methods (Sambrook *et al.* 1989). The DNA was digested using *Eco*RI + *Rsa*I and products were separated on 2 % (w/v) agarose gels to investigate the RFLP diversity amongst clones. DNA sequencing reactions, electrophoresis and acquisition of sequence data were carried out by MWG Biotech AG (London). Sequencing was performed from both ends of the vector insert.

Preliminary multiple sequence alignments were performed using ClustalW 1.8 (Smith *et al.* 1996). Homology studies were carried out using BLAST (blastn) (Altschul *et al.* 1997). GCG PILEUP (Anon. 2001) was used for sequence alignments before dendrogram analysis. TREEPUZZLE (Schmidt *et al.* 2002) was used to identify identical sequences and to estimate the transition:transversion ratio. PHYLIP (Felsenstein 2005) was used for DNADIST/NEIGHBOUR analysis. Pairwise comparisons were made amongst sequences with GCG GAP (Anon. 2001; Wisconsin Package Version 10.3, Accelrys, San Diego, CA). The nine new *E. betae* ITS sequences from this study have been deposited in the GenBank database under accession numbers DQ164432–DQ164440.

Results

RFLP patterns in ITS clones

Thirteen different *Eco*RI/*Rsa*I RFLP patterns were found after digesting 5–20 ITS clones per isolate (177 clones in total; Table 2). The most common RFLP pattern (with bands of approximately 1710, 1200 and 640 bp), designated class I and found in 119 out of 177 clones, was present in all powdery mildew isolates and was the sole type in isolates BB3, EB1-EB6 and FAR. These observations suggested that class I represented *Erysiphe betae* and that the other RFLP patterns were derived from companion or contaminant organisms. In general, fewer RFLP patterns were derived from glasshouse isolates compared with field isolates of the fungus. The identity of class I was confirmed after sequencing a small number of clones. Database searches revealed that class I was homologous to *E. betae* (Californian isolate from sugar beet; accession no. AF011290; score = 1178 bits, E value = 0.0) and was also extremely similar to an *E. heraclei* ITS sequence (AB104510; score = 1217 bits, E value = 0.0). Interestingly, the new sequence was not highly homologous to an isolate described as *E. betae* collected from sugar beet in Iran (acc. no. AB104516, Khodaparast *et al.* unpublished; Fig 1).

The other 12 RFLP patterns (Table 2) were rarer than class I, especially in the case of classes XI, XII, XIII and XIV, which were only represented by single clones. Representatives of each of these other RFLP classes were sequenced to determine whether they were also derived from *E. betae*, but with small

Table 1 – Name codes, collection sites and host plant identities of sugar-beet powdery mildew isolates used in this study

Isolate	Collecting location	Site	Host cultivar or line name
BB1	UK: Broom's Barn, Suffolk	Field	cv. 'Sandra'
BB2	UK: Broom's Barn, Suffolk	Field	cv. 'Sandra'
BB3	UK: Broom's Barn, Suffolk	Field	cv. 'Sandra'
EB1	UK: Broom's Barn, Suffolk	Glasshouse	Experimental line
EB2	UK: Broom's Barn, Suffolk	Glasshouse	Experimental line
EB3	UK: Broom's Barn, Suffolk	Glasshouse	Male-sterile breeding line
EB4	UK: Broom's Barn, Suffolk	Glasshouse	*Beta vulgaris* subsp. *maritima*
EB5	UK: Broom's Barn, Suffolk	Glasshouse	Experimental line
EB6	UK: Broom's Barn, Suffolk	Glasshouse	Male-sterile breeding line
PM11	UK: Broom's Barn, Suffolk	Glasshouse	Experimental line
C	UK: Claverley, Shropshire	Field	cv. 'Jessica'
S	UK: Shewsbury, Shropshire	Field	cv. 'Roberta'
WF	UK: Wainfleet, Lincolnshire	Field	cv. 'Humber'
WW	UK: Wicklewood, Norfolk	Field	cv. 'Wildcat'
FAR	USA: Fargo, North Dakota	Glasshouse	cv. 'ACH 9369'
KIM1	USA: Kimberly, Idaho	Field	Unknown commercial cultivar
KIM2	USA: Kimberly, Idaho	Field	Unknown commercial cultivar
ONT	USA: Ontario, Oregon	Field	cv. 'Hilleshög 2984Rz'

Table 2 – Approximate sizes of bands in RFLP patterns found after digesting powdery mildew ITS PCR products (previously cloned into pGEM) using *Eco*RI + *Rsa*I

n	Approximate band sizes (bp)
I	1710, 1200, 640
II	1700, 1180, 310
III	1700, 1190, 500, 230
IV	1720, 1200, 300
V	1680, 1180, 330, 220
VI	1610, 1160, 710, 310
VII	1620, 1170, 180, 130, 100
VIII	1660, 1190, 330, 170
IX	1240, 1070, 880
X	1620, 1160, 340, 130
XI	1920, 1280, 650
XII	1670, 1190, 230, 180, 160
XIII	1700, 1190, 330, 220, 110

changes that affected *Eco*RI or *Rsa*I sites. They were found to contain sequences that were significantly different from class I and included ITSs homologous to those from the phylloplane yeast-like fungi *Rhodotorula acheniorum* (GenBank accession no. AB03812), *Sporobolomyces roseus* (AY015438) and an *Epicoccum* sp. (AJ279463). These genera have previously been identified growing on sugar-beet leaf surfaces (Thompson *et al.* 1993). Several clones contained sequences homologous to the ITS from the powdery mildew microparasite and potential biocontrol agent, *Tilletiopsis washingtonensis* (AF294696). This appears to be the first published evidence to suggest the presence of a *Tilletiopsis* species on sugar beet, or in *E. betae* colonies. Further investigation would be required to demonstrate whether, if viable *Tilletiopsis* is present, it has any effects on *E. betae*.

The presence of the non-*E. betae* ITS sequences verified the necessity of selecting from amongst cloned PCR products to correctly identify *E. betae* products before sequencing.

Sequence variation within ITS clones

To investigate the diversity amongst *Erysiphe betae* ITSs, 23 class I clones, comprising at least one representative per original isolate, were sequenced. Fifteen of the 23 clones, BB1-1 (i.e. isolate BB1, clone 1), BB2-3, BB3-2, EB1-1, EB2-2, EB3-5, EB4-4, EB5-2, EB6-1, PM11-1, C-5, S-3, S-7, WF-13 and ONT-12, all contained identical sequences. This group of clones included UK and North American isolates, suggesting that there are no major differences between isolates from the two countries. Clones FAR-2, FAR-8, KIM1-1, KIM1-6, KIM2-2, KIM2-7, ONT-4 and WW-14 differed from the above common sequence by various single base substitutions (alignments not shown), and again, were derived from UK and North American isolates. The new *E. betae* ITS sequences have been deposited in the GenBank database under accession numbers DQ164432–DQ164440.

Dendrogram analyses

Preliminary analyses were done using all the ITS sequences of members of *Erysiphales* available from GenBank to ensure

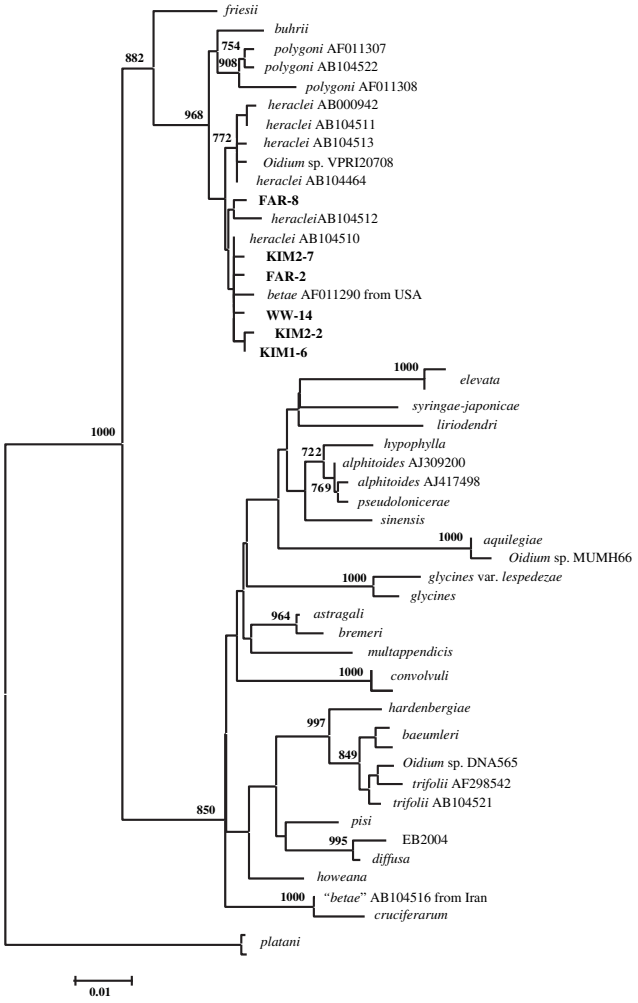

Fig 1 – DNADIST/NEIGHBOR analysis of 51 *Erysiphales* rDNA ITS1, 5.8 S and ITS2 region sequences (BS values shown where >700/1000). Unless otherwise stated, all species are *Erysiphe*. DNA database accession numbers are shown on the tree to distinguish multiple sequences from the same species, and are listed for all sequences used in Table 3. Six out of the nine new sugar-beet powdery mildew sequences, identified by clone name, are included in the analysis and are in boldface. Two of the nine new sequences were identical to *E. heraclei* sequence AB104510 over the length used, and so are not included separately in the analysis.

that all related sequences were used. After elimination of more distantly related sequences and of unnecessary duplicates, a final group of 51 unique 577 nucleotide sequences (Table 3) covering exclusively the entire ITS1, 5.8 S and ITS2 region was obtained. The polymorphisms that made three of the new *Erysiphe betae* sequences distinct were outside this 577 nucleotide section. Over the length of the sequence alignment used for the dendrogram analyses, two of these three sequences (EB1-1 and KIM1-1) were identical to *E. heraclei* sequence AB104510. This left six of the new *E. betae* sequences available for dendrogram analysis.

The alignment was used for NJ analysis (DNADIST and NEIGHBOUR) with the transition:transversion ratio (Ts/

Tv = 1.27) estimated by TREEPUZZLE. The dendrogram produced, which was bootstrapped to 1000 replicates, is shown in Fig 1, and a tree of similar topology was also obtained by ML analysis (DNAML; results not shown). *E. platani* was chosen as the outgroup because preliminary analyses had shown it to be the nearest species outside the group being analysed.

The new *E. betae* sequences formed a cluster with one of the previously published *E. betae* sequences (AF011290; from the USA). We conclude from this that the same fungus causes sugar-beet powdery mildew in the UK and the USA. The complete 51-sequence ITS dataset fragmented into two large clusters, supported by a BS value of 1000/1000, with the UK and North American sequences together in one major cluster, and the Iranian *E. betae* sequence (AB104516) in the second major cluster. The Iranian sequence formed a group with an isolate of *E. cruciferarum* collected from *Arabidopsis thaliana* (Adam *et al.* 1999) and is unrelated to the UK and

Table 3 – List of powdery mildew rDNA ITS sequences used in the phylogenetic analysis

Fungus	Isolate/designation	Host plant	Country of origin	GenBank accession no.
Erysiphe sp.	EB2004	*Phaseolus vulgaris*	Brazil	AY739109
E. alphitoides	GF3	*Quercus robur*	France	AJ309200
E. alphitoides	ascomyc4	*Quercus robur*	Germany	AJ417498
E. aquilegiae	VPRI 20820	*Aquilegia* sp.	Australia	AY452800
E. astragali		*Astragalus* sp.	Iran	AB104515
E. baeumleri	YNMH12852-5	*Vicia cracca*	Japan	AB015919
E. baeumleri	YNMH12360-12	*Vicia amoena*	Japan	AB015933
E. betae		*Beta vulgaris*	USA	AF011290
E. betae		*Beta vulgaris*	Iran	AB104516
E. betae	FAR (clone 2)	*Beta vulgaris*	USA (this study)	DQ164434
E. betae	FAR (clone 8)	*Beta vulgaris*	USA (this study)	DQ164435
E. betae	KIM1 (clone 6)	*Beta vulgaris*	USA (this study)	DQ164437
E. betae	KIM2 (clone 2)	*Beta vulgaris*	USA (this study)	DQ164438
E. betae	KIM2 (clone 7)	*Beta vulgaris*	USA (this study)	DQ164439
E. betae	WW (clone 14)	*Beta vulgaris*	UK (this study)	DQ164433
E. bremeri		*Alhagi* sp.	Iran	AB104463
E. buhrii		*Acanthophyllum* sp.	Iran	AB128924
E. convolvuli		*Convolvulus arvensis*	Iran	AB104518
E. convolvuli		*Convolvulus arvensis*	USA	AF011298
E. cruciferarum	UEA1	*Arabidopsis thaliana*	USA	AF031283
E. diffusa		*Glycine max*	Brazil	AY739112
E. elevata		*Catalpa bignonioides*	UK	AY587012
E. elevata		*Catalpa bignonioides*	USA	AY587014
E. friesii var. *dahurica*		*Rhamnus japonica*	Japan	AB000939
E. glycines var. *glycines*	MUMH14S	*Lespedeza thunbergii*	Japan	AB015923
E. glycines var. *lespedezae*	MUMH13S	*Lespedeza cuneata*	Japan	AB015921
E. heraclei		*Panax schin-seng*	Japan	AB000942
E. heraclei		*Bifora testiculata*	Iran	AB104464
E. heraclei		*Conium maculatum*	Iran	AB104510
E. heraclei		*Daucus* sp.	Iran	AB104511
E. heraclei		*Eryngium caucasicum*	Iran	AB104512
E. heraclei		*Pimpinella affinis*	Iran	AB104513
E. howeana		*Oenothera biennis*	USA	AF011301
E. hypophylla	VPRI 22120	*Quercus robur*	Japan	AF298544
E. liriodendri		*Liriodendron tulipifera*	USA	AF011302
E. multappendicis		*Berberis vulgaris*	Iran	AB104520
E. platani		*Platanus racemosa*	USA	AF011311
E. platani		*Platanus ocidentalis*	Australia	AF073349
E. pisi		*Lathyrus latifolius*	USA	AF011306
E. polygoni		*Polygonum* sp.	Iran	AB104522
E. polygoni		*Polygonum arenastrum*	USA	AF011307
E. polygoni		*Rumex crispus*	USA	AF011308
E. pseudolonicerae	MUMH86	*Cocculus trilobus*	Japan	AB015915
E. sinensis	VPRI 20272	*Castanea crenata*	Korea	AF298545
E. syringae-japonicae		*Syringa vulgaris*	Japan	AB015920
E. trifolii		*Trifolium pratense*	Iran	AB104521
E. trifolii	VPRI 22166	*Trifolium pratense*	Swizerland	AF298542
Oidium sp.	DNA565	*Eustoma grandiflorum*	Japan	AB079855
Oidium sp.	MUMH66	*Lycopersicon esculentum*	Japan	AB032483
Oidium sp.	VPRI 20708	*Convolvulus erubescens*	Australia	AF154328
O. hardenbergiae	VPRI 19879	*Hardenbergia* sp.	Australia	AY450959

North American *E. betae*. The morphology and host range of the Iranian isolate is not known (Khodaparast, pers. comm.). Without extensive morphological, host range and molecular studies comparing a larger number of isolates from Iran and UK/USA, no meaningful conclusions can be drawn, and further work is needed.

Two representatives of *E. heraclei* (AB104510 and AB104512) were also present in the *E. betae* cluster. Other *E. heraclei* ITSs were also closely related to the new sugar-beet powdery mildew sequences, and were grouped in a second cluster (BS value 772/1000) with an *Oidium* sp. (VPR120708) isolated from *Convolvulus erubescens* (*Convolvulaceae*) in Australia (Cunnington *et al.* 2003). The presence in this group of a powdery mildew fungus from *Convolvulus* was not expected, but Cunnington *et al.* (2003) found that the *Oidium* sp. was morphologically similar to *E. heraclei* and concluded that *Convolvulus* was an 'accidental host' (Blumer 1967) of *E. heraclei*.

The three *E. polygoni* sequences, together with one from *E. buhrii* (host-range *Caryophyllaceae*), formed a separate group from the *E. betae*/*E. heraclei* cluster, supported by a BS value of 968/1000. The pairwise comparisons (Table 4) showed that the new sequences, with the *E. heraclei* group, form a coherent unit with a nucleotide identity of approximately 99.0 % or more.

Discussion

The nomenclature of sugar-beet powdery mildew has not yet been standardised in the literature, with the fungus still being named *Erysiphe betae* in Europe and Asia, and mostly as *E. polygoni* in the USA. Therefore, the first objective of our study was to elucidate whether the same, or different, fungi caused powdery mildew on sugar beet in the UK and USA. The technique used was based on analysis of polymorphisms in the rDNA ITS sequences and has been widely used in many similar studies, e.g. on European and North American isolates of *E. symphoricarpi* on snowberry (Kiss *et al.* 2002), and European and North American isolates of *Oidium neolycopersici* on tomato (Kiss *et al.* 2005). The majority (15/23) of ITSs sequenced in our study were identical and were derived from both UK and North American isolates of the fungus. Only single base substitutions were present in the remainder of the sugar-beet powdery mildew ITSs, but they were still more than 99 % similar. All the new sequences formed a strongly supported cluster in our dendrogram analyses, showing for the first time that UK and North American sugar-beet powdery mildew isolates are conspecific.

The next question we sought to answer, was whether the name *E. betae* (as used in Europe and Asia) or *E. polygoni* (as largely used in North America), was most suitable for sugar-beet powdery mildew. The differences in nomenclature relate to different approaches to powdery mildew taxonomy. In North America, a broad species concept, based exclusively on ascomatal morphology without consideration of host range specialisation or anamorph characteristics (Salmon 1900), formed the basis of powdery mildew taxonomy. Whereas in Europe, a narrower concept including host range data and anamorph characteristics has been followed (Blumer 1933; Braun 1987; Braun *et al.* 2002), and isolates formerly included under the aggregate species *E. polygoni sensu* Salmon (1900; Blumer 1967) have been named as separate species. The relationship of the new sequences to *E. polygoni* was more distant than expected; instead our sequences clustered with those from *E. heraclei*, forming a separate group. We conclude from these findings, the known narrow host range of sugar-beet powdery mildew and its morphological description, that the name *E. polygoni* is not appropriate for the fungus and that *E. betae* should be used instead.

The close relationship of *E. betae* with *E. heraclei*, also alluded to by preliminary BLAST searches of the sequence databases, was not surprising. Morphologically, *E. betae* and *E. heraclei* share several similarities: both produce conidia singly, both have chasmothecial appendages that are simple or irregularly branched in a coral-like manner, and both produce three to five spores per ascus. Apart from host range, there are only small differences between the two, including the smaller conidium and chasmothecium size of *E. heraclei*. *E. heraclei* is restricted to *Apiaceae*, although there is one report of its presence on *Hedera helix* (*Araliaceae*; Braun 1995) and on *Convolvulus* (*Convolvulaceae*; Cunnington *et al.* 2003). To date, it has not been reported on *Amaranthaceae*.

The close similarity of ITSs from *E. betae* to those of *E. heraclei* could be explained in three ways: (1) sugar beet is sometimes an 'accidental host' (Blumer 1967) for *E. heraclei*; (2) *E. betae* and *E. heraclei* are different species that happen to have identical ITS sequences (Cunnington *et al.* 2003); or (3) they are the same species. The first hypothesis would have to be tested by inoculating powdery mildews from *Apiaceae* hosts onto sugar beet, especially using *E. heraclei* isolates from the type host *Heracleum sphondylium*. The second and third hypotheses could be tested by inoculating isolates between the *Amaranthaceae* and *Apiaceae*, combined with a close study of their morphology and of other molecular characteristics, e.g. sequence variation within the β-tubulin gene. Wyand & Brown (2003) used this technique on the cereal powdery mildew fungus *Blumeria graminis*, but amplification of such single-copy genes from a minute amount of starting material may be difficult (Cunnington *et al.* 2004). Cunnington *et al.* (2003) found that the ITS sequences of two powdery mildew isolates collected from *Viburnum tinus* were identical to a previously published sequence attributed to *E. viburni* obtained from *V. opulus*, and also to a sequence attributed to *E. hedwigii* from *V. lanata*. This, together with the extremely close morphological similarity and the common host genus of *E. viburni* and *E. hedwigii*, led Cunnington *et al.* (2003) to suggest that the two species are probably conspecific. To fully resolve whether *E. betae* and *E. heraclei* are conspecific, comparisons would have to be made using ITS sequence data from European *E. heraclei*, above all isolated from the type host *H. sphondylium*. *E. betae*, and *E. heraclei*, are barely distinguishable morphologically, and, taking into consideration the ITS sequence results, it seems that the only major remaining difference between the two species is their host ranges.

Since the *Erysiphales* are obligate biotrophs and exist in intimate and specific associations with their host, it has been suggested that their phylogeny is closely linked with the phylogeny of their hosts (Braun 1987, 1995). To investigate this, Matsuda & Takamatsu (2003) constructed phylogenetic trees for the powdery mildew genus *Golovinomyces*

Table 4 – Nucleotide identity (by GCG GAP) amongst sequences of *Erysiphe* from sugar beet and some related isolates

		KIM1-6	ONT-4	KIM2-2	EB1-1	FAR-2	KIM2-7	WW-14	FAR-8	KIM1-1	AB104510	AF011290	AB104464	AB104514	AB104513	AB000942	AB104511	AF154328	AB104512	AB128924	AB104522	AF011307	AF011308
Erysiphe betae	KIM1-6	*	99.8	99.7	99.7	99.5	99.5	99.5	99.4	99.4	99.7	99.5	99.0	99.5	99.0	99.0	99.3	99.3	99.0	98.5	98.2	98.6	98.0
E. betae	ONT-4		*	99.8	99.8	99.7	99.7	99.7	99.5	99.5	99.8	99.7	99.2	99.5	99.2	99.2	99.3	99.3	99.2	98.5	98.4	98.7	98.1
E. betae	KIM2-2			*	99.7	99.5	99.5	99.5	99.4	99.4	99.7	99.5	99.0	99.3	99.0	99.0	99.1	99.1	99.0	98.3	98.2	98.6	98.0
E. betae	EB1-1				*	99.8	99.8	99.8	99.7	99.7	100.0	99.8	99.3	99.7	99.3	99.4	99.5	99.5	99.3	98.6	98.5	98.9	98.3
E. betae	FAR-2					*	99.7	99.7	99.5	99.5	99.8	99.7	99.2	99.5	99.2	99.2	99.3	99.3	99.2	98.5	98.4	98.7	98.1
E. betae	KIM2-7						*	99.7	99.5	99.5	99.8	99.7	99.2	99.5	99.2	99.2	99.3	99.3	99.2	98.5	98.4	98.7	98.1
E. betae	WW-14							*	99.5	99.5	99.8	99.7	99.2	99.5	99.2	99.2	99.3	99.3	99.2	98.5	98.4	98.7	98.1
E. betae	FAR-8								*	99.4	99.7	99.5	99.0	99.3	99.0	99.0	99.1	99.1	99.3	98.3	98.2	98.6	98.0
E. betae	KIM1-1									*	99.8	99.7	99.3	99.7	99.2	99.2	99.5	99.5	99.2	98.6	98.4	97.6	97.0
E. heraclei	AB104510										*	99.8	99.3	99.7	99.4	99.4	99.5	99.5	99.2	98.6	98.5	98.9	98.2
E. betae	AF011290											*	99.1	99.5	99.2	99.2	99.3	99.3	99.0	98.4	98.5	98.8	98.0
E. heraclei	AB104464												*	99.7	99.5	99.3	99.5	99.5	99.0	98.3	98.2	98.5	97.8
E. heraclei	AB104514													*	99.8	99.7	99.8	99.8	99.3	98.6	98.5	98.8	98.1
E. heraclei	AB104513														*	98.8	99.7	99.7	98.9	98.5	97.6	98.5	97.9
E. heraclei	AB000942															*	99.8	99.5	98.9	98.3	97.6	98.5	97.9
E. heraclei	AB104511																*	99.7	99.1	98.5	98.5	98.6	97.9
Oidium sp. VPRI 20708	AF154328																	*	99.1	98.5	98.4	98.6	97.9
E. heraclei	AB104512																		*	98.6	98.1	98.5	97.7
E. buhrii	AB128924																			*	98.8	99.0	98.3
E. polygoni	AB104522																				*	99.3	98.5
E. polygoni	AF011307																					*	98.7
E. polygoni	AF011308																						*

using ITS sequence data and compared them with phylogenetic trees of their hosts, mainly members of *Asteraceae*. They found that there was co-speciation of *Golovinomyces* with its hosts, but that there had also been host-jumping events onto species in different plant families. *E. betae* and all the fungi closely grouped with it in our dendrogram analysis, except *E. heraclei*, have hosts in subclass *Caryophyllidae* of the dicotyledons: *E. betae* on *Amaranthaceae*; *E. buhrii* on *Caryophyllaceae*; and *E. polygoni* on *Polygonaceae*. The *Apiaceae* host family of *E. heraclei* is unrelated to the above plant taxa, and is placed in the subclass *Rosidae*. A host-jumping event onto the *Apiaceae* would seem the most likely explanation.

The third part of our study was to search for evidence of a second species causing powdery mildew on sugar beet that might explain the contradictions in published morphological descriptions of sugar-beet powdery mildew conidiogenesis. Should a second species also be able to infect sugar beet, this would be highly significant from a resistance breeding perspective. Powdery mildew resistance has been identified and characterised in sugar beet, including that controlled by major genes (Lewellen & Schrandt 2001; Janssen *et al.* 2003) which is likely to be highly specific against *E. betae*. Previously, Hull (1949, 1971) recorded conidia forming in long chains, whereas others (e.g. Weltzien 1963) found that conidia matured singly. Whether the conidia are solitary or catenate is one of a number of features used in powdery mildew taxonomy (Braun *et al.* 2002). However, conidia that are normally produced singly can sometimes adhere together abnormally in chains under conditions of high relative humidity (Cook *et al.* 1997). In our study, all 18 powdery mildew isolates contained identical ITSs (or had single base substitutions), and no molecular evidence was found for a second powdery mildew fungus of sugar beet.

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